(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: 0 292 846
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 88107910.7

(22) Date of filing: 18.05.88

(51) Int. Cl.[4]: C11C 3/04 , A61K 31/23 , A23D 5/00 , A23L 1/30

Claim for the following Contracting State: ES + GR.

(30) Priority: 28.05.87 IT 2070087

(43) Date of publication of application: 30.11.88 Bulletin 88/48

(84) Designated Contracting States: AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: INNOVA DI RIDOLFI FLORA & C. S.a.s.
Via Lamarmora, 14
I-50121 Firenze(IT)

(72) Inventor: Cornieri, Franco
Via Lamarmora, 14
I-50121 Firenze(IT)
Inventor: Di Fulvio, Walter
Via Lamarmora, 14
I-50121 Firenze(IT)

(74) Representative: Minoja, Fabrizio
Studio Consulenza Brevettuale Via Rossini, 8
I-20122 Milano(IT)

(54) **A process for the extraction of polyunsaturated fatty acid esters from fish oils and pharmaceutical and/or dietetic compositions containing said esters.**

(57) A process for the extraction of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid esters from crude fish oils, by means of transesterification with ethanol and H_2SO_4 and two-step molecular distillation.

EP 0 292 846 A2

A PROCESS FOR THE EXTRACTION OF POLYUNSATURATED FATTY ACID ESTERS FROM FISH OILS AND PHARMACEUTICAL AND/OR DIETETIC COMPOSITIONS CONTAINING SAID ESTERS

The present invention relates to a process for the preparation of a mixture of fatty acid alkyl esters having a high concentration in eicosapentaenoic and docosahexaenoic acids, starting from fish oils of various origin, and to pharmaceutical and/or dietetic compositions containing said mixture.

Moreover, the process according to the invention is suited to deodorize and deacidify said oils, in view of any dietetic and/or alimentary use.

Polyunsaturated fatty acids are known to play two important roles in human physiology: a structural role, as constituents of cell membrane phospholipids, and a functional role, as Prostaglandin precursors.

In fact, fatty acids of the α -linolenic acid family have a basic role in development and function of brain, retina and gonads, as well as the formation of PGI_3 and TxA_3, which are factors of paramount importance for the anti-platelet aggregating activity.

Among these, particularly important are the long chain members of the ω-3-family, i.e. eicosapentaenoic (20:5ω -3) or EPA and docosahexaenoic (22:6 ω - 3) or DHA acids, derivating from desaturation and elongation of α-linolenic acid, thanks to the intervention of the related enzymes (Δ -desaturase).

EPA, as the precursor of PGI_3 and TxA_3, exerts an anti-platelet aggregating activity and an anti-thrombotic effect which can be related to cyclooxygenase inhibition (aspirine-like effect) and/or to the competition with arachidonic acid for said enzyme, with an accordingly decreased synthesis of PGI_2 and TxA_2, which are known platelet aggregating agents.

DHA is the most important component of human lipids and brain and is present in high concentrations in synaptic membranes phospholipids, which may imply a role in nervous impulse transmission. Moreover, DHA being a structural element of platelet cell, it indirectly exerts an important role in anti-thrombotic action, due to the increase in platelet fluidity.

Recent studies evidenced a decrease in Δ-6 desaturase in man as the age goes on (after 35 years); said phenomenon causes thus an endogenous lack in the above mentioned acids, which therefore should be administered through diet or by means of suitable compositions. However, various practical difficulties opposed up to now a wide use of said acids in therapy or as alimentary integrators, which use on the other hand should be highly desirable, in view of the above reported biochemical and pharmacological considerations.

Said difficulties mainly relate to extraction of said acids from fish oils, purification and concentration to values convenient for the pharmaceutical use and deodorization thereof.

Even though a number of methods have already been proposed and disclosed, the above objects have still not been attained satisfactorily, as proved, inter alia, by the still restricted use of EPA and/or DHA, in spite of the remarkable potentialities thereof as drugs or alimentary integrators. The methods up to now known, which are based on different techniques such as degreasing, countercurrent extraction, urea addition, liquid chromatography, distillation, give rather low yields and products which easily deteriorate if exposed to air or light. Moreover, the major part of the known methods refers to purification of the only eicosapentaenoic acid, to the detriment of other useful unsaturated acids, such as DHA.

As an example, US Patent 4.377.526 discloses a process for purification of EPA or the esters thereof, which comprises treatment with urea, followed by fractional distillation. By said method, EPA percentages higher than 70% are obtained, whereas DHA is present only as a residue (3-5%).

More recently, US Patents 4.554.107 and 4.623.488 disclosed a purification method based on the technique known as molecular distillation: in this case, a deodorized fish oil is obtained which is enriched in EPA and DHA, in rather low yields (30%), due to the drastic conditions used.

A first object of the invention is therefore provided by a method for the extraction of DHA and EPA ethyl esters from crude fish oils in high yields, under conditions which can easily be applied on industrial scale, which give a stable and odourless product, which can be used in human therapy or as a dietetic and alimentary integrator.

A second object of the invention, in fact, is provided by pharmaceutical or alimentary compositions containing as the active ingredient a mixture of EPA and DHA ethyl esters, for the treatment or the prophylaxis of cardio-vascular diseases.

According to the present invention, it has been found that highly purified EPA and DHA ethyl ester mixtures can be obtained starting from crude fish oils, by subjecting them to a transesterification reaction with ethanol, in the presence of sulfuric acid, subsequent silica gel chromatography and two-steps molecular distillation, under controlled conditions.

The process of the invention can be easily carried out on industrial scale, and is characterized in that it

consists in a surprisingly low number of steps, if compared with the processes up to now known, which use crude fish oils as the starting material. Moreover, it should be stressed as particularly surprising that enriching in EPA and DHA and deodorizing are simoultaneously attained by a molecular distillation technique, which has been hitherto considered merely for the purpose of deodorization, with completely different operative parameters.

In fact, the above cited US patents carry out three-steps molecular distillation on long chain unsaturated acid triglycerids, using drastic temperature conditions (up to 260-300 °C), operating in the presence of two additives (glycerol and monooleylglycerid) in order to fluidize the liquid to be distilled.

On the contrary, the process object of the present invention is characterized in that it is carried out on the ethyl ester mixture instead of the triglycerides: moreover operative conditions are much milder (only two steps at a lower temperature) and give higher yields, mainly in DHA which is known to be less stable.

According to the invention, deodorization is effected in the first step and the products responsible for the bad smell are removed by the low temperature trap, upstream the pump. This allows to operate on crude oil not previously depurated, which, besides being effectively deodorized, is also deacidified, to make it suited for alimentary use.

A preferred EPA and DHA source consists in oils deriving from working of "blue fish", such as anchovies, sardines, cods, mackerels, herrings and the like.

The oil is diluted with ethanol, then refluxed in the presence of catalytic amounts of concentrated sulfuric acid. After extraction with hexane, the transesterification mixture is subjected to silica gel chromatography, then to a two-step molecular distillation process, with a vacuum of about 10^{-3} mm Hg and at an evaporation temperature ranging from 65-70 °C to 105-125 °C and condenser at 5 °C.

The product obtained as a distillation residue has and EPA+DHA content higher than 65% and the DHA:EPA ratio, which generally depends on the starting oil, is about 3:2.

According to an alternative process of the invention, the ethanolic solution obtained by transesterification can be treated with urea, in order to remove the salts of fatty acids of lower unsaturation. However, said further step generally is not necessary, since in the major part of cases the only molecular distillation is sufficient to attain the desired effects.

According to a further aspect of the process of the invention, it is also possible to obtain docosahexaenoic acid having an assay as high as 85-95%, according to the starting fish oil used. For this purpose, the ester mixture from the silica gel chromatography is dissolved in acetone and the solution slowly cooled to -40 °C. The formed precipitate is then filtered, the solvent is removed under reduced pressure and the residue is subjected to molecular distillation, which is carried out in two steps, always at 10^{-3}torr, but at higher temperatures, namely 80/100 °C for the first step and 105/125 °C for the second step.

The product obtained by the process of the invention proved to be particularly convenient for pharmaceutical use, in form of appropriate pharmaceutical compositions. In fact, a favourable synergism was evidenced between EPA and DHA, such as to give a therapeutical effectiveness higher than that of the single components. The pharmaceutical compositions of the invention will be prepared by means of techniques and excipients conventionally used for active ingredients in form of oils, as described in "Remington's Pharmaceutical Sciences handbook", Hack Pub. Co., N.Y. USA. Preferred administration routes are the oral and the parenteral ones, whereas posology will generally range form 500 to 5.000 mg of EPA and DHA ethyl ester mixture obtained by the inventive process, depending on pathology and conditions of the patient to be treated. Anyway, higher dosages are not controindicated, since the active ingredient is almost non-toxic. The same mixture can be used as dietetic or alimentary integrator, optionally diluted with other appropriate vegetal oils.

In fact, the mixture obtained by the process of the invention is particularly convenient for the prophylaxis of diseases related to platelet hyperaggregation conditions, since it is completely free from linolenic acid derivatives which are precursors of arachidonic acid and accordingly of PGE_2 and TxA_2 which are factors able to oppose and make void the favourable pharmacologic properties deriving from the production of PGI_3 and TxA_3, induced by EPA, DHA and derivatives thereof.

The following example further illustrates the invention without limiting it.

EXAMPLE

a) 80 kg of fish oil was dissolved in 50 l of ethanol containing 5% conc. sulfuric acid. The whole was refluxed under nitrogen for 8 hours, then cooled; ethanol excess was removed and the volume was doubled with water. At this moment an extraction was carried out with appropriate amounts of hexane. The hexane

solution, after repeated washings with water, was chromatographed on a silica gel 100 column to remove impurities. The solution from this column was freed from n-hexane under vacuum, to obtain a yield of about 65 kg of esterified products. Control can be effected by G.C. (gas chromatography)

b) The product from step a) was subjected to double-step molecular distillation, under a vacuum of about 10^{-3} mm Hg, at an evaporation temperature of 65-70 °C and condenser temperature of 5 °C.

Percentages of the fatty acids present in the oil before and after the treatment, determined by G.C., are reported hereinbelow.

Fatty acids	%* Ethyl esters on crude product	%* Ethyl esters on final product
$C_{14:0}$	8.4	0.1
$C_{16:0}$	16.0	1.7
$C_{16:1\omega7}$	9.8	0.3
$C_{18:1\omega9}$	9.9	3.0
$C_{18:1\omega7}$	3.1	1.0
$C_{20:5\omega3}$	9.6	29.8
$C_{22:5\omega3}$	1.0	4.1
$C_{22:6\omega3}$	10.7	39.9

Claims

1. A process for the extraction of eicosapentaenoic and docosahexaenoic acid ethyl esters from fish oils, which process comprises transesterification of the fish oil with ethanol, in the presence of sulfuric acid, subsequent extraction of the mixture with hexane, silica gel chromatography and two-step molecular distillation.

2. A process as claimed in claim 1, in which molecular distillation is carried out at a temperature of 65-70 °C under a vacuum of about 10^{-3} mm Hg.

3. A process as claimed in claims 1 or 2, in which the mixture from transesterification is treated with urea before being subjected to the following operative steps.

4. A process for the extraction of docosahexaenoic acid ethyl ester from fish oil, comprising transesterification of the fish oil with ethanol, in the presence of sulfuric acid, subsequent extraction of the mixture with hexane, silica gel chromatography, treatment of the residue in acetone cooled to -40 °C, filtration, evaporation of acetone and two-steps molecular distillation at 10^{-3}torr, the first step at a temperature of 80-100 °C and the second step at a temperature of 105-125 °C.

5. A product obtained by the processes of claims 1-3, consisting at least by 65% in eicosapentaenoic and docosahexaenoic acid ethyl esters.

6. A product as claimed in claim 5, in which docosahexaenoic acid ethyl esters to eicosapentaenoic acid ethyl esters ratio is 3:2, respectively.

7. Pharmaceutical and/or alimentary compositions containing the product as claimed in claims 5-7, in admixture with appropriate excipients.

Claims for the following Contracting States: GR, SP

1. A process for the extraction of eicosapentaenoic and docosahexaenoic acid ethvl esters from fish oils, which process comprises transesterification of the fish oil with ethanol, in the presence of sulfuric acid, subsequent extraction of the mixture with hexane, silica gel chromatography and two-step molecular distillation.

2. A process as claimed in claim 1, in which molecular distillation is carried out at a temperature of 65-70 °C under a vacuum of about 10^{-3} mm Hg.

3. A process as claimed in claims 1 or 2, in which the mixture from transesterification is treated with urea before being subjected to the following operative steps.

4. A process for the extraction of docosahexaenoic acid ethyl ester from fish oil comprising transesterification of the fish oil with ethanol, in the presence of sulfuric acid, subsequent extraction of the mixture with hexane, silica gel chromatography, treatment of the residue in acetone cooled to -40 °C, filtration, evaporation of acetone and two-steps molecular distillation at 10^{-3}torr, the first step at a temperature of 80-100 °C and the second step at a temperature of 105-125 °C.